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Megophrys maosonensis Bourret, 1937 (Anura: Megophyridae) - A new India record from Talley Valley Wildlife Sanctuary, Arunachal Pradesh, India

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Abstract

Megophrys longipes maosonensis, described from Mao Son, Vietnam is long considered to be a junior synonym of *Megophrys major* species complex. However, recently, it has been resurrected from its synonymy into a valid species, with its distribution restricted to Vietnam and the adjacent areas of China. In 2015, we have made a sample collection of *Megophrys* from Talley Valley Wildlife Sanctuary in Arunachal Pradesh on which we performed DNA barcoding using fragments of mitochondrial 12S rRNA and 16S rRNA. This has resulted in the specimen being identified as *M. maosonensis* making it a new record for India.

Keywords: DNA Barcoding, Eastern Himalayas, New Country Record, Talley Valley, 12S, 16S

Introduction

Mahony *et al.*, (2018) have studied the cryptic diversity of the Indian *Megophrys major* species group (except *M. maosonensis*, due to the non-availability of this species in India) and erected 4 new species from the country, thereby taking the total species count under *Megophrys* to 17 (Frost, 2019). Bourret (1937) erected *Megophrys longipes maosonensis* but soon after synonymised it with *M. major* (Bourret (1942). *M. maosonensis* belongs to the *M. major* species group, which till recently was considered a junior synonym of *M. major* until Chen *et al.*, (2016) resurrected it into a valid species with new name combination, subsequently supported by Mahony *et al.*, (2017; 2018). Chen *et al.*, (2016) have also reported *M. maosonensis* from multiple locations in Vietnam and China.

During the period of 2015-18, a number of surveys were conducted by one of the authors (BJS) to Talley Valley Wildlife Sanctuary (TWS) in Arunachal Pradesh, the easternmost state of India, during which a number of amphibians were collected including some *Megophrys* frogs. A *Megophrys* species so far endemic to China, *M. pachyproctus* has recently been reported from Talley Valley Wildlife Sanctuary (Saikia & Sinha, 2018). Among these collections, is a *Megophrys* specimen that morphologically bore a close resemblance to *M. major*, with some differences [upper jaw not white, body not robust but slender, indistinct bands on hindlimbs, the inter-orbital triangular blotch with lighter central region and a distinct dot-like blotch on the snout] and, therefore warranted molecular analysis to establish its specific identity. DNA barcoding on fragments of 12S and 16S rRNA, established it to be *M. maosonensis*, thereby confirming the first record of this species from India.

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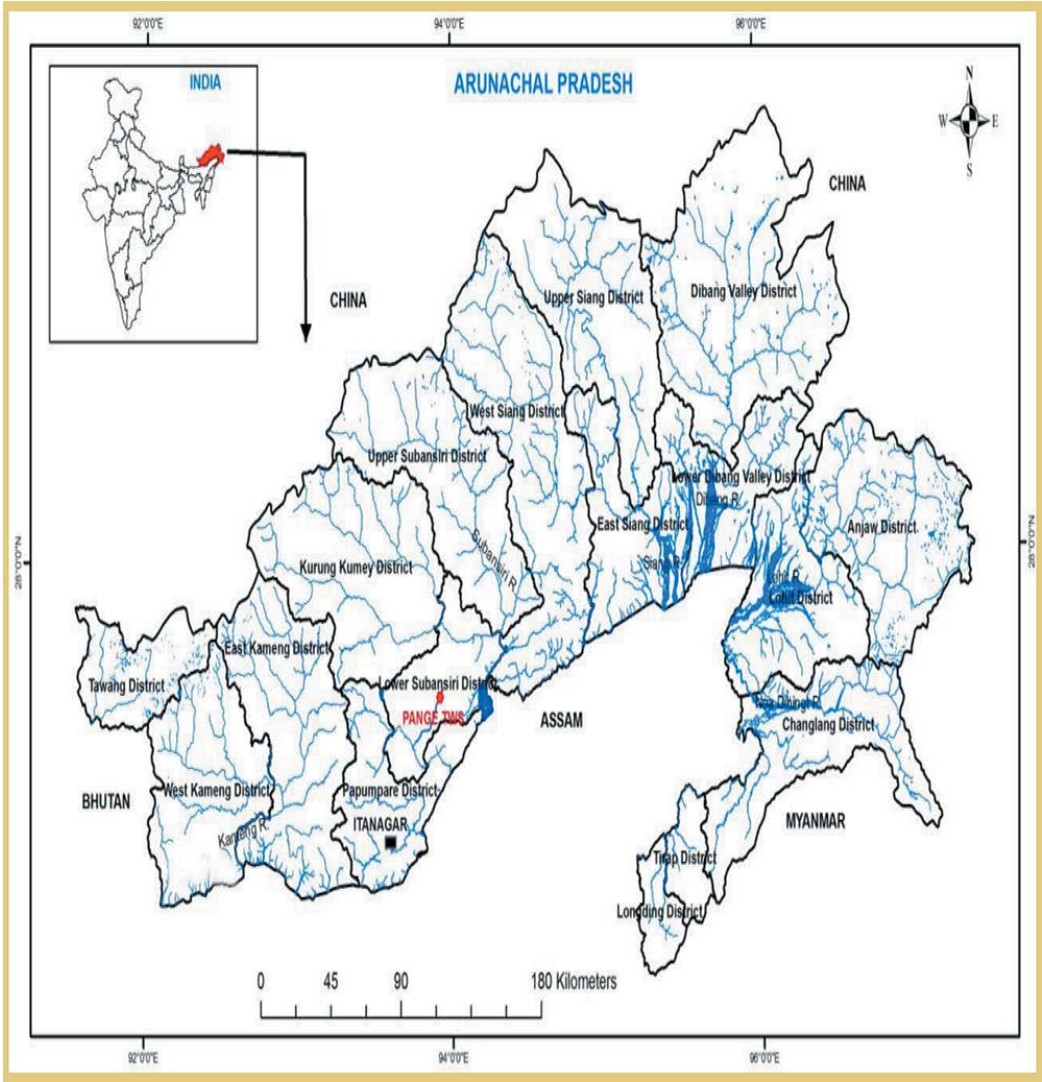
Material and Methods

Sample Collection

The specimen (Figure 1A-E) was collected on 5 November, 2015, between 17:00 to 20:00 hrs from Pange [27.5485 N; 93.8989 E; 1804 m] in Talley Valley Wildlife Sanctuary, located in the Lower Subansiri district of Arunachal Pradesh, India (Map 1). All measurements (in millimetres) were done with a Mitutoyo™ digital calliper. The measurements used are SVL (Snout Vent length), HL (Head Length), HW (Head Width), MN (Mandible Nostril distance), MFE (Distance of Mandible to the Front of Eye), MBE (Distance of Mandible to the Back of Eye), IFE (Distance between the Front of Eyes), IBE (Distance between the Back of Eyes), IN (Inter-Narial distance), EN (Distance from Eye to Nostril), SN (Distance from Snout tip to Nostril), EL (Eye Length), SL (Snout Length), TYD (Horizontal Tympanum Diameter), TYE (distance from anterior border of tympanum to the posterior orbital border), IUE (Minimum distance between the upper eyelids), UEW (maximum width of the upper eyelid), HAL (Hand length from the base of inner metacarpal tubercle to the tip of third finger), FLL (Forearm Length from the elbow



Map 1. Map showing collection locality of *M. maosonensis* from Pange, TWS, Arunachal Pradesh, India..

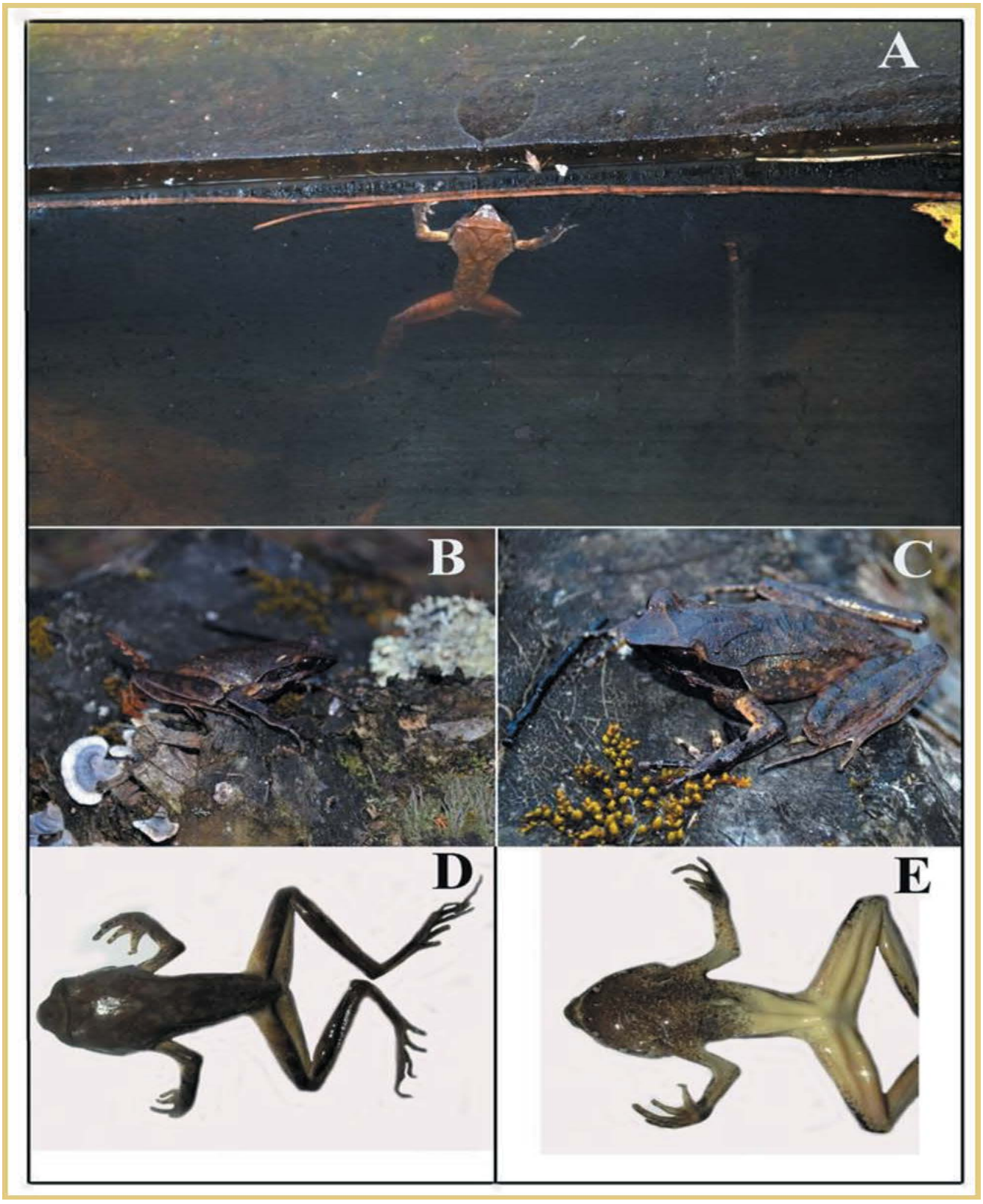


Figure 1. The specimen of *Megophrys maosonensis* collected from TWS, Arunachal Pradesh, India. A, B, C – Live animal; D, E – preserved specimen.

joint to base of the inner metacarpal tubercle), FL (Femur Length), TL (Tibia Length), TFOL (Length of Tarsus & Foot), FOL (Foot Length, from inner metatarsal tubercle to the tip of fourth toe), TTA (Tibio-Tarsal Articulation). Dissection of the specimen was carried out to ascertain its sex. GPS coordinates were recorded using GARMIN Oregon 550. The specimen is registered (V/A/NERC/1370) and deposited in the National Zoological Collection of the Zoological Survey of India, Shillong.

DNA Extraction, PCR Amplification and Phylogenetic Analysis

Genomic DNA was extracted using Phenol-chloroform isoamyl alcohol method (Bowtell and Sambrook, 2002) and independent PCRs were carried out using the universal primers to amplify the partial fragment of 12S rRNA (Kocher *et al*, 1989) and 16S rRNA (Palumbi, 1991) genes to obtain sequences for species level identification. The amplified PCR products were cleaned up using the Exo-SAP treatment and Sanger sequencing was performed on Genetic Analyzer 3730 (Applied Biosystems, USA) using the Big Dye terminator cycle sequencing kit v3.1 (Applied Biosystems, USA). The raw sequences were cleaned up using Sequencher version 5.4.6 (www.genecode.com) and matched using BLAST (Basic Local Alignment Search Tool). The most homologous sequences present on NCBI were downloaded and phylogenetic tree was re-constructed based on lowest BIC value in MEGA X (Kumar *et. al.*, 2018) following Maximum Likelihood method with Kimura 2-parameter and Tamura 3-parameter models for 12S rRNA and 16SrRNA genes, respectively.

Result

Molecular Analysis

The specimen yielded sequences of 406bp (12S rRNA) and 558bp (16S rRNA) lengths and homology search showed a 95.37 to 97.52 % similarity with *Megophrys maosonensis* with query cover 99 to 100 % of 12Sr RNA and 16S rRNA genes, respectively.

The maximum likelihood trees for the two genes also corroborated findings of BLAST and also grouped specimen with the *Megophrys maosonensis* with strong bootstrap support (Figures 2 and 3). The estimated bootstrap values higher or equal to 70% usually correspond to probabilities higher or equal to 95% which means that the topology is close to real, giving a quantitative measurement of certainty of the assignment of a sample to a particular species. The sequences

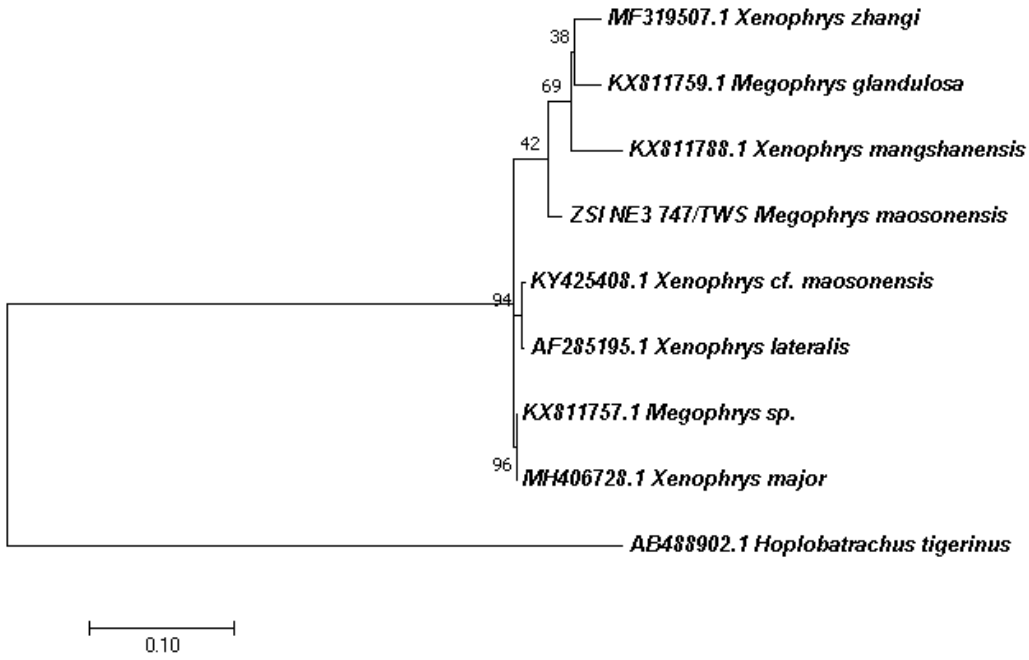


Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method based on 16S rRNA gene.

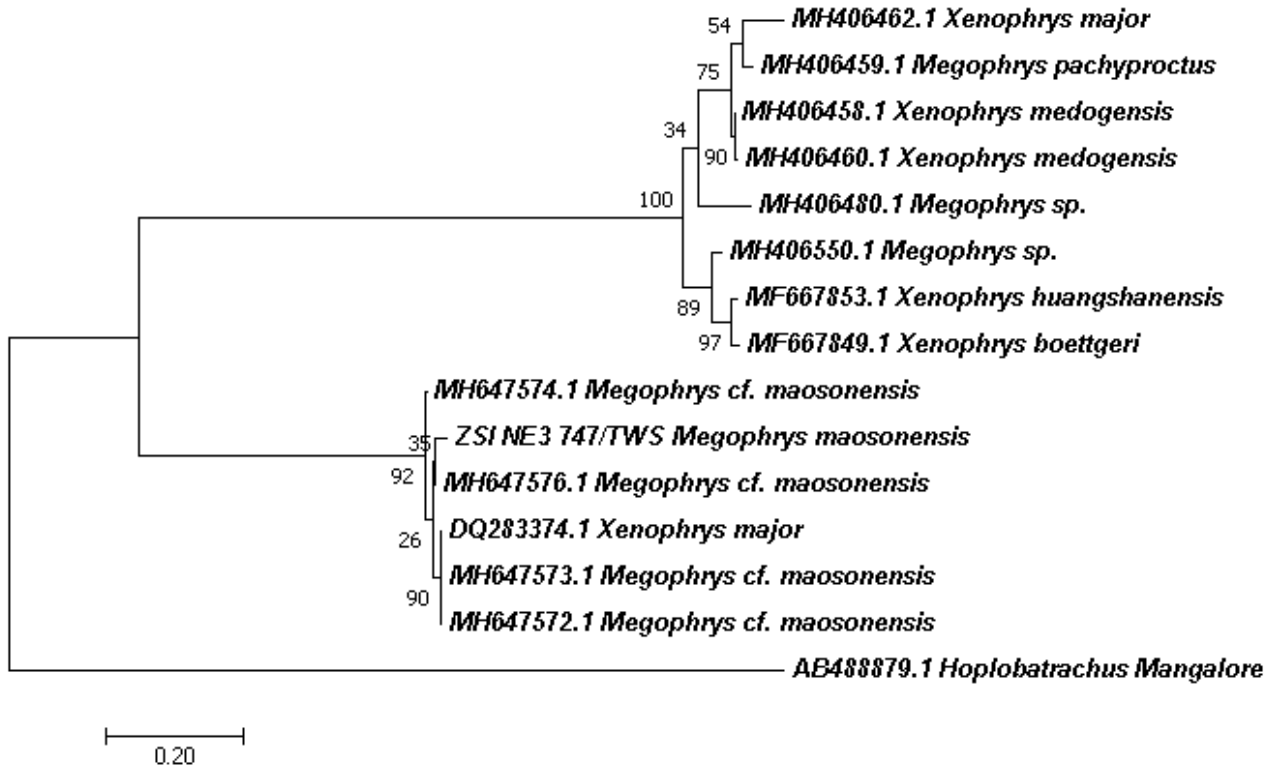


Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method based on 12S rRNA gene.

generated for *Megophrys maosonenesis* were deposited to the GenBank/NCBI (Accession MH423717 for 12S rRNA and MH423746 for 16SrRNA gene).

Morphological Diagnosis: A medium sized male frog (SVL-53.95 mm; *see* Table 1); body slender (Figure 1B&C). Head broader than long, snout pointed. Nostril closer to eye. Vomerine teeth present, located on the inner side of choanae; tongue entire and rounded. Tympanum is separated from the eye by a distance larger than its horizontal diameter. Supra-tympanic fold prominent. Pupil vertical; eye diameter is more than inter-orbital distance. Dark triangular patch between the eyes of which the central region is lighter. A dark blotch on the snout. A V-shaped tubercle on the occiput. A pair of tubercles on the dorso-lateral sides, extended only to half of the dorsum. Limbs banded. Fingers free,

Table 1. Morphometric measurements of *M. maosonensis* from TWS, Arunachal Pradesh, India

Sl. No.	Characters	*M. maosonensis*	Sl. No.	Characters	*M. maosonensis*
1	Sex	Male	13	SN	4.27
2	SVL	53.95	14	SL	6.85
3	HL	16.84	15	TYD	3.00
4	HW	22.62	16	TYE	4.80
5	MN	14.09	17	IUE	5.50
6	MFE	11.68	18	UEW	5.24
7	MBE	6.26	19	HAL	13.70
8	IFE	11.07	20	FLL	12.74
9	IBE	16.72	21	FL	27.97
10	IN	7.22	22	TL	32.47
11	EN	2.98	23	TFOL	44.07
12	EL	6.00	24	FOL	28.34

with relative length I≤II=IV<III; I slightly shorter than II. Hindlimbs slender, tibia longer than femur. Dorsum darker with extensive mottling of a lighter shade; in preserved condition, dark with certain dark markings (Figure 1D). Ventrum light in colour; anteriorly speckled with dark markings (Figure 1E).

Natural History: The lone specimen was spotted in a water tank, located about half kilometre from the forest guard camp at Pange in Talley Valley Wildlife Sanctuary that served as the drinking water source for the camp. The surrounding area is forested with mixed tropical evergreen vegetation. The associated herpetofauna of the area include *Leptobrachium bompu, Megophrys pachyproctus, Rhacophorus burmanus, R. rhodopus, Ptyas nigromarginata, Sphenomorphus maculates and Japalura* sp.

Distribution: China [Yunnan] and Vietnam [Cao Bang province, Vinh Phuc province and Lao Cai province] (Chen *et. al*., 2016); India [Arunachal Pradesh] (this report).

Discussion

Arunachal Pradesh is one of the least explored areas in terms of amphibian diversity. In recent years, however, studies on this group of fauna has led to a number of new India reports and as well as new species descriptions (Mathew and Sen, 2009; Sondhi and Ohler, 2011; Mahony *et al.,* 2013; Borah *et al,* 2013; Sarania *et al.,* 2015; Saikia *et al.,* 2017; Mahony *et al*., 2018; Saikia *et al.,* 2018).

The taxonomic history of *M. maosonensis* is interesting. As mentioned in the introductory section, Bourret (1937) erected *M. longipes maosonensis*; however, soon he synonymised it with *M. major* in 1942. After 74 years, Chen *et al.,* (2016) resurrected it into a valid species rank based on collection of specimens from multiple locations in China and Vietnam and put it in the *Megophrys major* Species Group (MMSG).

Identification of species belonging to cryptic species groups based on morphology have severe limitations due to overlapping morphological characters and create taxonomic confusion (Mahony *et al*., 2018). Mahony *et al.,* (2013) have highlighted the difficulties taxonomists face while working on the *Megophrys* group as majority of species were described from China in various Chinese languages and as such, literature has remained obscure in English. Access to literature and types are some of the reasons that have warranted the workers to resort to molecular studies to ascertain proper specific identity of species belonging to such cryptic groups. Therefore, this report of *M. maosonensis* in India (Arunachal Pradesh) is based on both morphological as well as molecular studies carried out by the authors. This report, thus, extends the distribution of the species to Arunachal Pradesh in India, which is further westward and northwards of its known distribution range.

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